Tyrosine Kinase Inhibitors: From Rational Design to Clinical Trials

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Abstract: Protein kinases play a crucial role in signal transduction as well as in cellular proliferation, differentiation, and various regulatory mechanisms. The inhibition of growth related kinases, especially tyrosine kinases, might provide new therapies for diseases such as cancer. The progress made in the crystallization of protein kinases has confirmed that the ATP-binding domain of tyrosine kinases is an attractive target for drug design. Three successful examples of drug design at Novartis using a tyrosine kinase as a molecular target are described. PKI166, a pyrrolo[2,3,-*d*]pyrimidine derivative, is a dual inhibitor of both the EGFR and the ErbB2 kinases. The compound entered clinical trials in 1999, based on its favorable preclinical profile: potent inhibition of EGF-mediated signalling in cells, *in vivo* antitumor activity in several EGFR overexpressing xenograft tumor models in nude mice, long-lasting inhibition of EGF-stimulated EGFR autophosphorylation in tumor tissue, good oral bioavailability in animals, and no prohibitive *in vitro* and *in vivo* toxicity findings. The anilino-phthalazine derivative PTK787/ZK222584 (Phase I, co-developed by Schering AG, Berlin) is a potent and selective inhibitor of both the KDR and Flt-1 kinases with interesting anti-angiogenic and pharmacokinetic properties (orally bioavailable). STI571 (Glivec^{TM}, Gleevec^{TM}), a phenylamino-pyrimidine derivative, is a potent inhibitor of the Abl tyrosine kinase, which is present in 95% of patients with chronic myelogenous leukemia (CML). The compound specifically inhibits proliferation of v-Abl and Bcr-Abl expressing cells (including cells from CML patients) and shows anti-tumor activity as a single agent in animal models at well-tolerated doses. Pharmacologically relevant concentrations are achieved in the plasma of animals (oral administration). Promising data from phase I and II clinical trials in CML patients (98% haematological response rate in Phase I) support the fact that the STI571 represents a new treatment modality for CML. In addition, potent inhibition of the PDGFR and c-Kit tyrosine kinases also indicates its possible clinical use in solid tumors.
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Key words: tyrosine kinase inhibitors; Glivec^{TM}; pharmacophore model; ATP-competitive inhibitors; PKI166; PTK787; ZK224584; STI571

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1. TYROSINE KINASES ARE ATTRACTIVE ANTICANCER TARGETS

Protein tyrosine kinases (PTKs) play a crucial role in signal transduction pathways that regulate a number of cellular functions (*e.g.*, differentiation, proliferation), both under normal cell function as well as under disordered conditions. Due to the enormous progress that has been made in the past few years regarding identification of the human genome, combined with advances made in molecular, biochemical and cell biology technologies, as well as synthetic chemistry, computer-assisted molecular modeling, and crystallography, the number of receptors (*e.g.*, receptor protein tyrosine kinases) being used as molecular targets in drug discovery has greatly increased. In the search of low molecular weight therapeutic agents against cancers (and other disease indications) PTKs have become attractive targets for the following reasons:

- In each cell, roughly 200–300 protein kinases are present.
- Protein kinases are found upstream/downstream of epidemiologically relevant oncogenes or tumor suppressor genes (which kinase is a good target?).
- The commonality as well as diversity among ATP-binding sites of kinases allows the building of pharmacophore models and rational drug design.
- There is the possibility of a step-wise concept validation at the biochemical, cellular and intact animal level as proof of concept in the clinic.

Since the initiation of the first drug discovery programs in the tyrosine kinase field about 15 years ago, an ever-increasing number of PTKs (receptor and non-receptor PTKs) has been selected as viable molecular targets. An overview of important relevant kinase targets is given in Table I.

Initially, inhibition of PTKs by ATP-directed compounds was considered less advantageous than substrate based inhibitors due, among other factors, to the assumption that the catalytic domain among protein kinases is highly conserved and selectivity with ATP-competitive inhibitors would be very difficult to achieve. The progress made in the crystallization of protein kinases—more than 40 crystal structures of protein kinases, in most cases complexed with ATP-site directed inhibitors,[1] are described in the literature—has confirmed that the ATP-binding domain of tyrosine kinases is indeed an attractive target for drug design.

Table I. Protein Kinases in Human Cancer

Overexpression of growth factor receptors
EGFR, HER2, HER3, HER4 in breast, ovary, lung, head & neck and glioblastoma
FGFR in lung, ovary and breast
PDGFR in glioblastoma
IGF-1R in solid tumors
Overexpression of growth factors
TGF-α in carcinoma overexpressing the EGFR
PDGF-BB expression in glioblastoma
VEGF expression for neo-vascularization (angiogenesis and metastasis)
Altered protein kinase levels and/or activities
Bcr-Abl in CML (95%), ALL (15%)
c-Met in renal carcinoma
c-Kit in gastric cancers (GIST)
Cancers with Onc Ras (about 30–50%) have deregulated PKC,
Raf-kinase (bladder, colon, lung, breast)
Deregulation of cell cycle by CdKs

In the absence of the X-ray structure of a kinase (*e.g.*, EGFR protein tyrosine kinase), pharmacophore models have been constructed and further refined with structure-activity relationships (SAR) studies.[2,3,4] These models are now generally applicable and have enabled researchers to exploit structure-based drug design approaches to increase potency and/or selectivity within a lead series.

Over the past years, numerous reviews have covered the design, synthesis and biological evaluation of ATP site-directed inhibitors of PTKs (for recent reviews, see Refs).[1,5,6,7,8,9] From the optimization programs of a variety of lead structures (*e.g.*, quinazolines,[10,11] pyrido[d]- and pyrimido[d]-pyrimidines,[12,13,14] pyrazolo[d]-pyrimidines,[15] pyrrolo[d]pyrimidines,[16] phenylamino-pyrimidines,[17,18] 1-oxo-3-aryl-1H-indene-2-carboxylic acid derivatives,[19] and substituted indolin-2-ones[20,21] or natural products such as staurosporine), several compounds have passed the hurdles of preclinical development and have entered clinical development. Currently, there are at least eleven ATP-competitive inhibitors in various phases of clinical evaluation (Table II).

In the following part, we describe three successful examples of, drug design at Novartis using a tyrosine kinase as a molecular target culminating in drugs which have entered clinical trials after broad preclinical evaluation.

2. *PKI166—A DUAL EGFR/c-ErbB-2-TYROSINE KINASE INHIBITOR*

About 15 years ago, when research in the signal transduction field was initiated in many pharmaceutical companies, the epidermal growth factor receptor (EGFR) was chosen as a "prototype" drug discovery target. The EGF family of type I receptor tyrosine kinases consists of four members: HER1 (c-ErbB-1/EGFR); HER2 (c-ErbB2/neu); HER3 (c-ErbB3); and HER4 (c-ErbB4). All four members are close homologs and share common structural elements, like an extracellular ligand-binding domain and a cytoplasmic tyrosine kinase domain. Much evidence has accumulated over recent years that the EGFR and its family members are strongly implicated in the development and progression of numerous human tumors including breast, lung, colorectal, ovarian, prostate, and head and neck. Refer to Voldborg *et al.*[22] for a recent review. Based on the clinical epidemiology and feasibility, the EGFR has been viewed as an ideal target in drug discovery programs, and many low molecular weight compounds have proven to show a promising *in vitro* and *in vivo* preclinical profile. Currently, there are five EGFR tyrosine kinase inhibitors in clinical

Table II. Small Molecule Tyrosine Kinase Inhibitors in Clinical Trials

Inhibitor	*Company*	*Target kinase(s)*	*Status*
STI571 (Glivec™)	Novartis	Abl (PDGFR, c-Kit)	Phase III (p.o.)
ZD1839 (Iressa)	AstraZeneca	EGFR	Phase III (p.o.)
OSI-774	Roche/OSI	EGFR	Phase III (p.o.)
PKI166	Novartis	EGFR, HER2	Phase I (p.o.)
CI1033	Pfizer/Warner-Lambert	EGFR, HER2(3,4) (irreversible inhibitor)	Phase I (p.o.)
EKB-569	Wyeth-Ayerst	EGFR, HER2 (irreversible inhibitor)	Phase I
SU5416	SUGEN	VEGFR	Phase III (i.p.)
PTK787/ ZK224584	Novartis/Schering AG	VEGFR	Phase I (p.o.)
SU6668	SUGEN	PDGFR/VEGFR/FGFR	Phase I (p.o.)
ZD6474	AstraZeneca	VEGFR/EGFR	Phase I (p.o.)
CEP2583	Cephalon	Trk	Phase II

development (Table II). This includes three quinazoline-type compounds: ZD1839 (AstraZeneca, Phase III),[7,11,23] OSI-774 (CP-358,774, Roche/OSI-Pharmaceuticals, Phase II/III),[8,10,24] CI-1033 (irreversible inhibitor, PD-183805, Pfizer/Warner-Lambert, Phase I),[25,26] EKB-569 (Wyeth-Ayerst, Phase I),[27] and the pyrrolo-pyrimidine PKI166 (Novartis, Phase I).

Initially, pyrrolo[2,3-d]pyrimidines were identified in random screening as lead structures and further optimized using a pharmacophore model of the ATP binding site of the EGFR tyrosine kinase.[16] This optimization process led to the discovery of PKI166 (Fig. 1), a very potent dual inhibitor of the EGFR/erbB-2 tyrosine kinases.[28] Fig. 1 shows the putative binding mode of PKI166 docked in the ATP binding site of the EGFR kinase-model. The pyrrole NH of the inhibitor (corresponding to the 6-amino group of the adenine ring of ATP) together with the pyrimidine N1 atom form a hydrogen bond donor–acceptor system with two amino acids of the enzyme (Gln 767 and Met 769). These interactions position the phenol moiety of PKI166 in a hydrophobic pocket which is not exploited by ATP in tyrosine kinases. In addition, the hydroxy phenol group donate a hydrogen bond to Glu 738, a residue proximal to the pocket. On the other side of the inhibitor, the benzylic moiety occupies the region of the active site normally involved in binding the ribose group of ATP. Noteworthy is the possibility of hydrophobic contact between the phenyl ring and Cys 773. Cysteine at this position is a distinctive feature of the members of the ErbB family. The corresponding amino acid in the other protein kinases usually has a polar side chain. Thus, the binding model, through this putative Cys 773 hydrophobic interaction, provides an explanation for the high selectivity displayed by PKI166 towards the EGFR and ErbB-2 kinases (for more details of this and an alternative binding mode refer to Ref. 28).

PKI166 inhibits both the intracellular domain (ICD) of the EGFR and the HER2 tyrosine kinases with IC_{50} values in the low nanomolar range (Table III). Enzyme kinetic studies are in accordance with ATP-competitive inhibition of the EGFR tyrosine kinase. The compound shows a high degree of selectivity with respect to the inhibition of the serine/threonine kinases (CDK-1, PKC-α and PKA), but only moderate selectivity against some tyrosine kinases, inhibiting a number of them at sub-micromolar concentrations (*e.g.*, c-Abl, c-Src, KDR). However, a selectivity ratio of > 100 is still achieved for all tyrosine kinases tested (Table III).

As expected from the *in vitro* enzyme data, PKI166 inhibits signaling through the ligand-activated EGFR in cells (Table III). In a cell-based ELISA assay, EGF-induced autophosphorylation

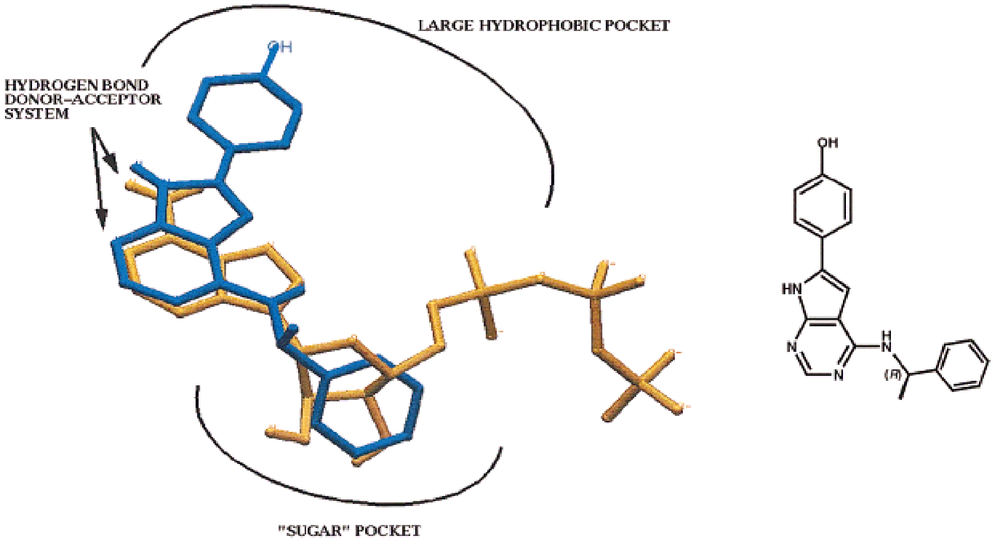


Figure 1. PKI166: Structure and mode of binding.

Table III. *In Vitro* Profile of PKI166

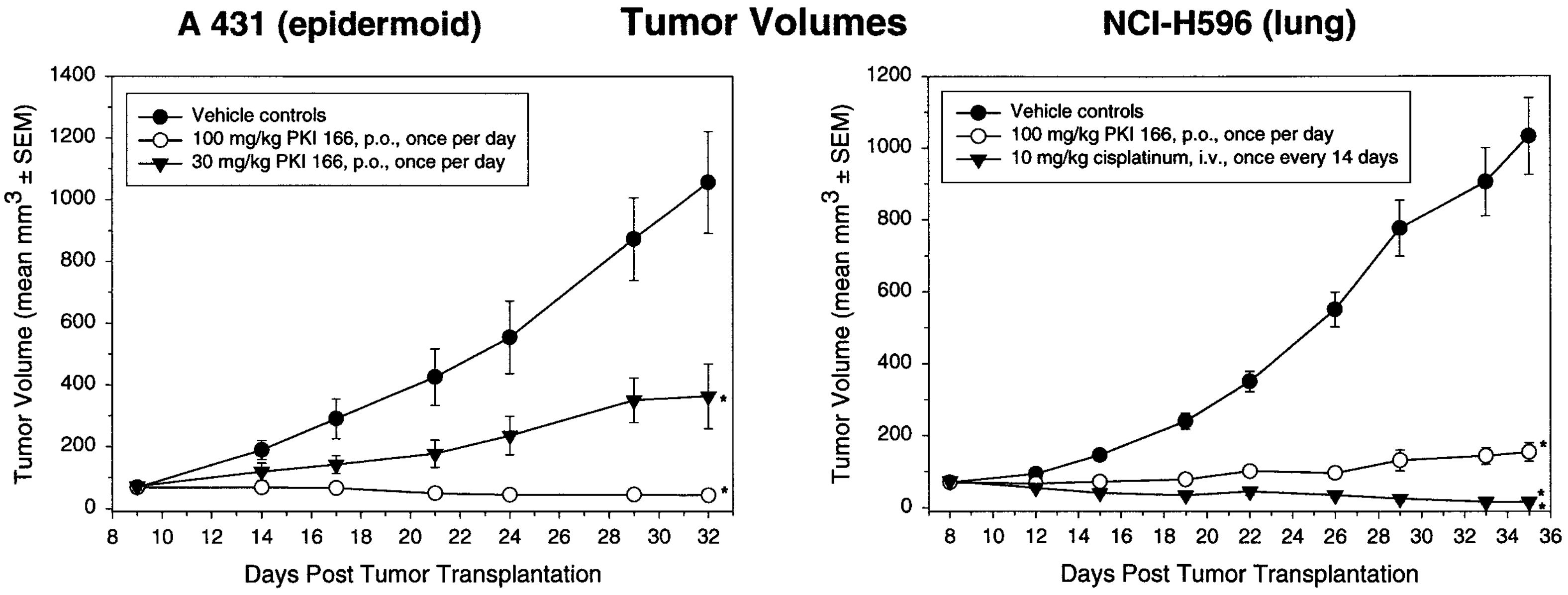
Kinase	***(IC_{50}, μM)***	***Intracellular phosphorylation***	***(IC_{50}, μM)***
HER1	0.001	EGFR (ELISA)	0.051
HER2	0.011	c-ErbB2/HER2	0.1–1
HER4	0.433	c-Kit	0.1–1
c-Abl	0.361	PDGF-R	> 10
c-Src	0.130	IGF-IR, Ins-R	> 10
GST-KDR	0.327	***Inhibition of c-fos***	(IC_{50}, μM)
Flt-1	0.962	EGF	0.1–1
c-Kit	2.210	bFGF	> 10
Flk	> 1	PMA	> 10
c-Met	> 1	***Inhibition of proliferation***	(IC_{50}, μM)
Tek	> 1	MK (EGF-dependent)	0.45
PKC-α	> 10	A431 (EGF-dependent)	0.26
CDK 1/2	> 10	H596 (EGF-dependent)	0.94
		SK-Br-2 (erbB2-dependent)	3.05
		BT-474 (erbB2-dependent)	1.29
		T24 (EGF-independent)	7.1

All IC_{50} values are averages of at least three determinations. Kinase assays were performed with γ-^{33}P-ATP and various acceptor substrates. Standard deviation for all assays was $< 50\%$. Properties of cell lines were taken from literature information or were determined experimentally.

of the EGFR was inhibited with an IC_{50} value of 51 nM. Western blot analysis using anti-phosphotyrosine antibodies gave similar results (IC_{50} value between 10 and 30 nM). Immunoblotting with anti-EGFR antibodies showed that the compound did not affect the overall level of EGFR. PKI166 also inhibited ErbB2 autophosphorylation in two human breast carcinoma cell lines (SK-BR-3 and BT-474) and in c-*erb*B-2 transfected NIH3T3 cells with IC_{50} values between 0.1 and 1 μM. In contrast, PKI166 did not affect PDGF-stimulated autophosphorylation of the PDGFR in cells ($IC_{50} > 10$ μM). PKI166 effectively inhibited EGF-induced c-*fos* induction with an IC_{50} value between 0.1–1 μM, whereas c-*for* mRNA induction in response to FGF or PMA was much less sensitive to inhibition.

In cell proliferation assays, PKI166 selectively inhibits the growth of EGF-dependent BALB mouse keratinocytes, EGFR-overexpressing A431 and NCI-H596 cell lines and the ErbB-2-overexpressing SK-BR-3 and BT-474 cell lines (Table III). In contrast, inhibition of EGF-independent cell lines such as T24 bladder carcinoma cells occurred at significant higher concentrations.

The correlation between the responsiveness to growth inhibition and expression of the EGFR has also been confirmed at the *in vivo* level. When administered orally, PKI166 produced significant and dose-dependent antitumor effects in various EGFR-dependent xenograft models in nude mice (*e.g.*, human epidermoid A-431 carcinoma, lung NCI-H596 carcinoma, Fig. 2). As an example, against A-431 tumors a daily oral dose of 30 mg/kg of PKI166 produced a final T/C of 29%, whereas the higher dose of 100 mg/kg/day induced durable regressions. Both doses were well tolerated, and there was no mortality or significantly body weight loss (Figure 2).[29] High peak plasma and tumor concentrations of PKI166 were achieved following administration of a single 100 mg/kg oral dose to A 431 tumor-bearing athymic nude mice ($C_{max} = 9.6$ and 21.9 μM, respectively). Even 24 hr after dosing, substantial concentrations of the drug could still be detected in tumors ($C_{24\ hr} = 1.5$ μM), exceeding by a factor of 150 the IC_{50} for inhibition of autophosphorylation in A-431 cells (measured by Western blotting). Using two different methods (immunoblotting and immunohistochemistry), PKI166 was shown to hit the target *in vivo* by producing a complete and long-lasting inhibition of EGF-stimulated EGFR autophosphorylation in tumors. Even 8 hr post-treatment, EGFR autophosphorylation was still completely blocked (Fig. 3), and 24 hr post-treatment, the inhibition was still



Tumor Volumes

A 431 (epidermoid)

NCI-H596 (lung)

No dead animals, no body weight loss

Figure 2. Antitumor effects of PKI166 against EGFR-dependent xenografts in nude mice. **A431:** Tumor fragments of approximately 25 mg were implanted into the left flank of each female nude mouse ($n = 7$ per group). Treatment was started on day 9 after tumor transplantation. PKI166 was administered p.o. once per day at 30 or 100 mg/kg until 32 days post tumor transplantation. $^*P < 0.05$ *vs.* vehicle controls. **NCI-H596:** Tumor fragments of approximately 25 mg were implanted into the left flank of each male nude mouse ($n = 8$ per group). Treatment was started on day 8 after tumor transplantation. PKI166 was administered p.o. once per day at 100 mg/kg until 35 days post tumor transplantation. Cisplatinum was administered at 10 mg/kg on days 8 and 22. $^*P < 0.05$ *vs.* vehicle controls.

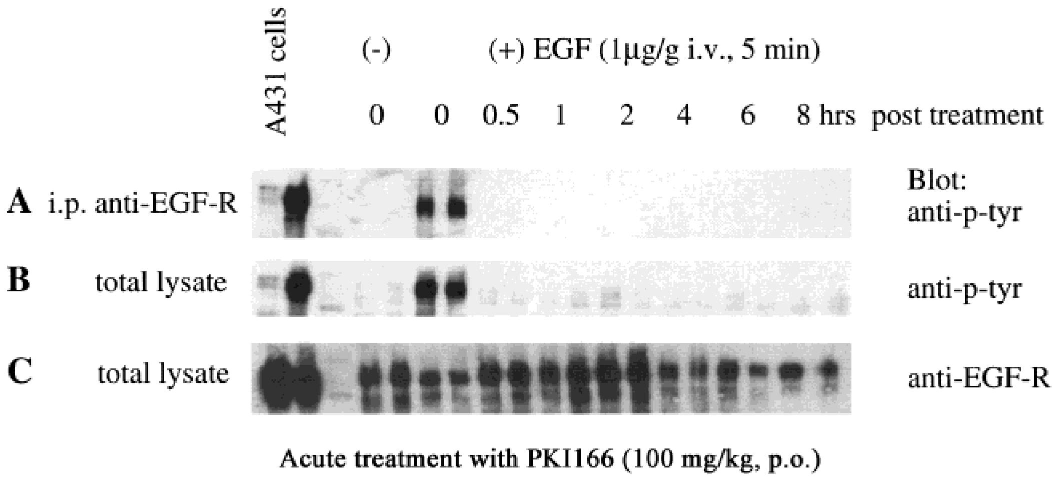

A431 cells
(-)
(+) EGF (1μg/g i.v., 5 min)
0 0 0.5 1 2 4 6 8 hrs post treatment
A i.p. anti-EGF-R
B total lysate
C total lysate
Blot:
anti-p-tyr
anti-p-tyr
anti-EGF-R
Acute treatment with PKI166 (100 mg/kg, p.o.)

Figure 3. Long-Lasting Inhibition of EGFR Phosphorylation in A431 Tumors by PKI166 ("Hitting the Target"). A431 tumor-bearing nude mice were administered PKI166 once orally (100 mg/kg) and at various times after the dose mice were injected with EGF. Five minutes after administration of EGF, tumor samples were extracted, immunoprecipitated with anti-EGFR antibodies and analyzed by Western blotting using anti-phosphotyrosine antibodies. In panel B, total lysates were analyzed by Western blotting using anti-phosphotyrosine antibodies. This membrane was stripped and reprobed with anti-EGFR antibodies.

$>50\%$.[30] These data indicate that alternative regimens beside once daily administration could also be feasible and are currently under investigation.

In addition, it could be demonstrated that oral administration of PKI166 either given alone or in combination with gemcitabine (administered i.p.) showed antitumor and antimetastatic activity in an EGFR-dependent orthotopic pancreatic carcinoma in nude mice leading to a significant increase in overall survival time. The therapeutic effects were mediated partly by inhibition of tumor-induced angiogenesis as shown by a significant reduction in tumor cell production of VEGF and IL-8 which correlated with a significant decrease in microvessel density and an increase in apoptotic endothelial cells.[31]

Based on current preclinical data and the expression of EGFR in human tumors, PKI166 is expected to be active against tumors associated with overexpression of or dysfunctional EGFR, including carcinomas of lung (NSCLC), breast, head and neck, prostate, ovary, urinary, bladder, gastrointestinal tract, and gliomas. PKI166 is currently in Phase I trials.

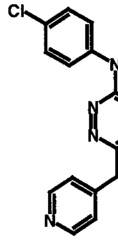
3. PTK787/ZK222584—A VEGFR TYROSINE KINASE INHIBITOR

Solid tumors cannot grow beyond a certain size until they establish a blood supply by inducing the formation of new vessels sprouting from existing host capillaries. The vascular endothelial growth factor (VEGF), a mitogen specific for vascular endothelial cells, is considered to play a key role in the angiogenic process and has been shown to be secreted by tumor cells and macrophages. The angiogenic signal is transmitted via cell surface receptors (*KDR* and *Flt-1*) located on the host vascular endothelium, which have intracellular tyrosine kinase activity. Inhibition of VEGF-induced angiogenic signals will selectively target the tumor-associated vessels and may reduce tumor-induced edema. Anti-angiogenic therapy through inhibition of the VEGF-mediated effects is expected to be safe and well tolerated in cancer patients. Therefore, the VEGFR family of tyrosine kinases are considered to be attractive targets for rational drug design.[32,33,34]

PTK787/ZK222584 (Fig. 4), (co-developed by Novartis/Schering AG, Berlin), potently inhibits recombinant VEGFR kinases and is the most selective VEGFR kinase inhibitor described (Table IV).[35] This profile can be rationalized on the basis of the putative binding mode of PTK787 to the ATP-binding site of VEGFR-2 (for details see Ref.).[36]

PTK787 readily penetrates cells and inhibits the autophosphorylation of VEGFR-2 in both HUVECs and KDR-transfected chinese hamster ovary cells.[37] It possesses good functional activity in cellular systems, inhibiting VEGF-mediated cell proliferation ($IC_{50} = 16$ nM), cell survival and cell migration ($IC_{50} = 58$ nM) in HUVECs. As expected for a selective VEGF inhibitor, it neither inhibited FGF-stimulated proliferation of HUVECs, nor the proliferation of cells not expressing VEGF receptors. Anti-angiogenic activity has been demonstrated *in vitro* through inhibition of growth factor-stimulated capillary sprouting from rat aorta ($IC_{50} = 680$ nM) (Table IV).

Following oral administration to mice (50 mg/kg), PTK787 is rapidly absorbed, giving peak plasma concentrations of about 30 μM, with concentrations remaining above 1 μM at 8 hr.[37] In accordance with this pharmacokinetic profile, the compound displays good efficacy by the oral route. Thus, in a growth factor implant model, PTK787 (12.5–50 mg/kg, p.o.) inhibited VEGF-induced vascularization of a subcutaneous implant in mice, quantified by measurement of the weight and blood content of the vascularized tissue surrounding the implant. In a model of malignant pleural effusion, a complication of advanced lung cancer, consistent with inhibiting VEGF-mediated events, PTK787 (50 mg/kg/day, p.o.) suppressed tumor vascularisation and markedly reduced the volume of pleural effusate in nude mice bearing lung tumors produced by human PC14PE6 adenocarcinoma cells.[38] Consistent with its anti-angiogenic activity. PTK787 possesses good efficacy in inhibiting the growth of VEGF-producing human carcinomas in number of murine xenograft models, with CWR-22 human prostate carcinoma being completely inhibited in some mice.[37] In the case of A-431 epithelial carcinoma, as well as inhibiting tumor growth, PTK787 (50 mg/kg p.o.) reduced the occurrence of microvessels and induced necrosis within the tumors. PTK787 (50 mg/kg/day p.o.) has also been shown to have anti-metastatic activity in an orthotopic



Succinate Salt

Formula (MG)	**$C_{20}H_{15}N_4Cl$ (346.82)**
• New Chemical Entity	**Anilino-Phthalazine**
• Synthesis	**6 Steps**
• No chiral centre	
• Status	**Phase I**

- **Potent inhibitor of VEGFR tyrosine kinases KDR and Flt-1 (IC_{50} ~ 30 - 100 nM).**
- **Selectively inhibits VEGF-mediated endothelial cell proliferation.**
- **Selectively inhibits VEGF-mediated angiogenesis in mice.**
- **Inhibits growth of s.c. and orthotopic tumors in nude mice.**
- **Reduces vascularity and blood flow in artery of renal tumors.**
- **Inhibits lung/lymph node metastasis in orthotopic rodent tumor models.**
- **No effect on healing of incisional wounds in mice and rats.**
- **High plasma levels after oral administration to mice and rats.**
- **Well tolerated: Acute MTD > 250 mg/kg, p.o.**
- **Despite c-Kit inhibition, no significant effects on blood or bone marrow cells**

Figure 4. Summary Preclinical Profile of PTK787/ZK222584.

Table IV. *In Vitro* Profile of PTK787/ZK222584

Kinase	***(IC_{50}, μM)***		
VEGF-R2/KDR	0.037	***Receptor autophosphorylation***	***(IC_{50}, μM)***
VEGF-R1/Flt-1	0.077	KDR	0.0034
VEGF-R2/Flk	0.270	PDGF-R	0.74
PDGF-R-β	0.580	c-Kit	< 0.1
c-Kit	0.730	c-ErbB2/HER2	> 10
FGF-R1	> 1	IGF-IR	> 10
FGF-R2	> 1	***Functional cellular assays***	***(IC_{50}, μM)***
c-Met	> 1	VEGF-induced HUVEC proliferation	0.016
Tek	> 1	bFGF-induced HUVEC proliferation	> 1
EGF-R	> 10	IL-2-induced hPBL proliferation	> 10
v-Abl	> 10	Serum-induced proliferation of A431	15
c-Src	> 10	Serum-induced rat aortic piece sprouting	0.68
PKC-α	> 10		

All IC_{50} values are averages of at least three determinations. Kinase assays were performed with γ-^{33}P-ATP and various acceptor substrates. Standard deviation for all assays was < 50%.

model of murine renal cell carcinoma, where both tumor growth and the number of metastases were inhibited in comparison with control animals.

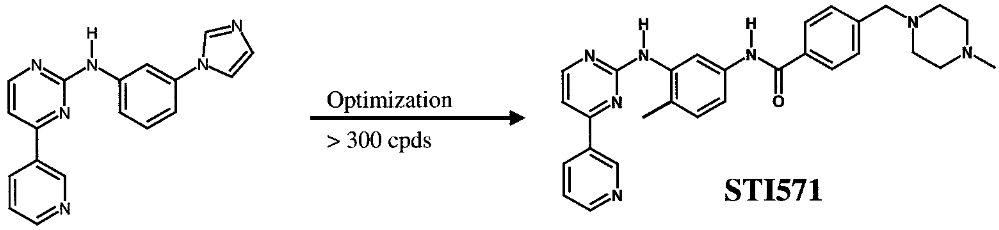
In terms of safety, PTK787 is well tolerated in animals and does not impair wound healing, as has been speculated for anti-angiogenic agents, nor does it have significant effects on leukocyte levels as might results from c-kit inhibition.[37] On the basis of its promising pre-clinical profile, PTK787/ZK222584 was selected for clinical evaluation and is currently in Phase I trials.

4. STI571[a]—A NEW TARGETED THERAPY FOR CML

Chronic myelogenous leukemia (CML) is a hematological stem cell disorder characterized by excessive myeloid proliferation.[39] Due to a reciprocal translocation between chromosome 9 and 22, the Philadelphia chromosome is formed. As a result of this translocation, a novel hybrid BCR gene is formed which produces a Bcr-Abl fusion protein with enhanced tyrosine kinase activity. This Bcr-Abl tyrosine kinase is present in 95% of patients with CML and 10–15% of adults suffering from acute lymphoblastic leukemia (ALL).[40] Due to this clear etiology, CML represents an ideal disease target for the use of a selective inhibitor of the Bcr-Abl kinase.

STI571 (Fig. 5) results from the optimization process of the phenylamino-pyrimidine lead class. Originally, phenylamino-pyrimidines were identified in the course of a random-screening as inhibitors against PKC-α (Fig. 5). Due to the ability to produce large number of compounds applying simple chemistry and the “drug-likeness” of the core structure, this class of compounds proved to be an excellent lead for the development of selective protein kinase inhibitors with high potency.[41,42] Exploitation of the diversity of various substituents at the 4-position of the pyrimidine-ring and the introduction of methyl group in 6-position of the phenyl ring (“flag methyl group”), led to compounds where the activity against PKC-α was completely lost, whereas the potency for the inhibition of the v-Abl and PDGFR tyrosine kinases was significantly increased. STI571 was selected for further development (Fig. 5).

[a] Glivec^{TM}, Gleevec^{TM}.



- Lead from random screening
    - PKC: IC50: 1 µM
    - Abl: IC50: >10 µM
    - PDGFR: IC50: >10 µM
- High potential for diversity
- Simple synthesis
- Drug-like properties
- ATP-competitive

- Introduction of *"Flag-methyl group"*
- Potent inhibition of Abl/PDGFR/Kit
- No activity on Ser/Thr kinases
- 10 Step synthesis
- Salts soluble in water
- Orally bioavailable
- ATP-competitive

Figure 5. STI571—from random screening to clinics.

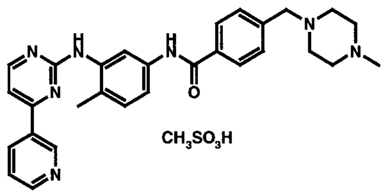
When STI571 was tested *in vitro* against a panel of protein kinases, it potently inhibited the v-Abl tyrosine kinase ($IC_{50} = 38$ nM), but was inactive against other tyrosine kinases (EGFR, KDR, Flt-1, c-Src, c-Met, *etc.*) and serine/threonine kinases (PKC, PKA).[43–45]

In cellular systems, the compound inhibited autophosphorylation of v-Abl ($IC_{50} = 0.25$ µM)[43] as well as the kinase activity of activated Abl fusion proteins such as p210bcr-abl, p185bcr-abl and TEL-Abl.[43,46,47] When tested against other tyrosine kinase, STI571 preferentially inhibited signal output from the ligand-activated PDGF receptor[44,45] and stem cell factor (SCF)-mediated events such as c-Kit autophosphorylation and MAP kinase activation in MO7e cells.[42,48] In contrast, signal transduction mediated by EGF, insulin, insulin-like growth factor I (IGF-I), fibroblast growth factor (FGF) and phorbol ester was insensitive to STI571.[44]

When tested for antiproliferative activity, STI571 showed selective growth inhibition of p185bcr-abl and TEL-Abl expressing cells.[46] Importantly, STI571 selectively blocked proliferation of Bcr-Abl positive CML and ALL cell lines, thereby inducing apoptosis.[47,49–51] Cell killing was also observed in fresh leukemic cells from Ph positive CML and ALL patients.[47,49] In contrast, STI571 had no effect on Bcr-Abl-negative ALL and AML primary blast cells.[47]

In vivo antitumor activity of STI571 after oral administration was demonstrated in nude mice injected with the Bcr-Abl-positive KU812 cell line derived from a CML patient in blast crisis. Since STI571 is rapidly absorbed and pharmacologically relevant concentrations are achieved in the plasma, continuous blockade of Bcr-Abl tyrosine phosphorylation in the tumor was achieved, resulting in tumor-free survival of mice that had been injected with KU812 cells.[52]

Based on the attractive preclinical profile of STI571, a dose escalating phase I trial was initiated in CML patients in mid 1998 which gave very promising results. When a daily dose of STI571 was given to patients who had failed to respond to interferon therapy, the compound was generally well tolerated and no maximally tolerated dose was identified in this study. At doses of 300 mg or greater, 97% of all patients had complete hematological responses (reduction of WBCs to normal), and a significant number of patients also achieved major cytogenetic responses (<35% Ph+ chromosomes).[53] High response rates have also been achieved in all phases of the disease (chronic phase, accelerated phase, blast crisis) in Phase II trials (91% hematological response rate in chronic



Phenylamino-pyrimidine

- **Formula (MG)** $C_{30}H_{35}N_7SO_4$ **(589.7)**
- **New Chemical Entity**
- **Synthesis** **8 Steps**
- **Achiral**
- **Water-soluble (Methanesulfonate salt)**

- **Potent inhibitor of the Bcr-Abl, PDGFR and c-Kit tyrosine kinases.**
- **Selective *in vitro* and *in vivo* activity against Bcr-Abl expressing cells.**
- **Selects for growth of benign progenitor cells from CML patients.**
- **Favourable PK profile in humans, allowing once daily oral treatment.**
- **Well tolerated in humans.**
- **Shows encouraging results in Phase I/II studies in CML. WBC returned to normal. Accelerated Phase trials ongoing.**
- **FDA approval May 2001.**

Figure 6. Summary data of STI571 (Glivec^{TM}).

phase) (unpublished results). These data raise hope that STI571 might replace bone marrow transplantation in CML and has the potential for cure of CML. STI571 received FDA approval May 2001.

4. OUTLOOK

In the last few years, competition in the field of signal transduction has become very intense. With the discovery of novel tyrosine kinases, the number of molecular targets, which are suitable for drug discovery projects has markedly increased. Thanks to the enormous progress that has been made in the crystallization of protein kinases, models for the binding of lead structures at the ATP-binding site have been developed and refined. This allowed rapid 3D screening of large compound libraries for lead identification and the rational design of highly potent and selective tyrosine kinase inhibitors. New techniques in chemistry (combinatorial chemistry, parallel synthesis, *etc.*) have accelerated the optimization process of lead structures.

A first wave of low molecular weight tyrosine kinase inhibitors has passed the hurdles of preclinical development and entered clinical trials. Clinical proof of concept (POC) has successfully been achieved with several of these drug candidates (*e.g.*, Abl kinase inhibitors STI571, EGFR kinase inhibitors ZD1839 or OSI-774), VEGFR kinase inhibitors SU5416 or PTK787/ZK224584). These encouraging clinical results will greatly stimulate further research in this area.

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